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The impact of the increased use of piperacillin/tazobactam on the selection of antibiotic resistance among invasive *Escherichia coli* and *Klebsiella pneumoniae* isolates

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SUMMARY

Objectives: Increasing antimicrobial resistance is related to the selective pressure exerted by antibiotic usage. This study evaluated the impact of the increased use of piperacillin/tazobactam (TZP) on the selection of antibiotic resistance.
Methods: From 1999 to 2010, *Escherichia coli* and *Klebsiella pneumoniae* invasive isolates obtained from hospitalized Korean children were analyzed in antibiotic susceptibility tests and subjected to characterization of the β-lactamase types. Antibiotic consumption data were also analyzed.
Results: Between January 1999 and December 2010, 409 invasive isolates of *E. coli* ($n = 170$) and *K. pneumoniae* ($n = 239$) were obtained. A rebound of extended-spectrum β-lactamase (ESBL) prevalence with an increase in total antibiotics was noted. Non-susceptibility to TZP was determined in 7.6% of *E. coli* isolates and 20.9% of *K. pneumoniae* isolates. Despite the increase in TZP usage, the overall prevalence of TZP resistance did not significantly increase over time, especially in *E. coli*. The mechanisms for TZP resistance included the presence of AmpC producers, possible TEM-1 hyperproducers, and multiple β-lactamases in individual organisms of a given isolate.
Conclusions: Replacement of only the antibiotic class appears to be insufficient to control antibiotic resistance, and continued efforts to decrease overall antibiotic pressure are needed, especially in highly endemic situations.

1. Introduction

Extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella pneumoniae* were first described in the 1980s, but are now recognized worldwide.[1] It is well-established that increases in antimicrobial resistance, including to β-lactams, can be ascribed to the selective pressure exerted by antibiotic usage, with the withdrawal of such pressure frequently used as an effective means of resistance reversal.[2,3]

To control ESBL prevalence in the Seoul National University Children's Hospital, a policy restricting the use of extended-spectrum cephalosporins (ESCs), replacing them with β-lactam/β-lactamase inhibitor combinations such as piperacillin/tazobactam (TZP) and ampicillin/sulbactam, has been instituted since 2002.[4] During a study carried out at our hospital from 1999 to 2005, the overall ESBL prevalence decreased, with no increase in TZP resistance determined among *E. coli* or *K. pneumoniae* isolates, despite a significant increase in the use of TZP.[4]

It has been reported that the clinical use of β-lactam/β-lactamase inhibitor combinations results in the selection of point mutants in TEM penicillinases resistant to inhibitors, referred to as inhibitor-resistant TEMs (IRTs).[5] These enzymes are generally susceptible to cephalosporins.[6] However, in the mid-1990s, complex mutant TEMs (CMTs) that combine ESBL and IRT mutations began to emerge among the TEM β-lactamases.[7,8] Moreover, the detection in ESBL assays of strains producing CMT-type β-lactamases may be difficult because of their high-level resistance to clavulanate. Another consequence of the increased use of β-lactam/β-lactamase inhibitor combinations is the emergence and spread of plasmids encoding AmpC β-lactamases.[9] However, a significant increasing trend in plasmid-mediated AmpC producers or in producers of any other specific type of

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ESBLs was not noted in our hospital, despite the increasing use of TZP throughout the above-mentioned study period.[4]

In this study, the impact of the increased use of TZP on the selection of antibiotic resistance, focusing on β-lactamase producers among invasive *E. coli* and *K. pneumoniae* isolates obtained from hospitalized Korean children, was evaluated, following an extended study carried out from 1999 through 2010. Antibiotic consumption and the prevalence of antibiotic resistance including ESBLs and TZP resistance were compared during four periods: 1999–2001 (period 1; pre-intervention), 2002–2003 (period 2; transition period), 2004–2006 (period 3; immediate post-intervention), and 2007–2010 (period 4; late post-intervention).

2. Materials and methods

2.1. Study setting and bacterial isolates

Our institute is a 300-bed, university-affiliated, tertiary hospital located in Seoul, Korea. *E. coli* and *K. pneumoniae* isolates from normally sterile body fluids were collected between January 1999 and December 2010 and kept at −70 °C. Species were identified using VITEK-GNI cards (bioMérieux, Hazelwood, USA).

2.2. Susceptibility to β-lactams

The antibiotic susceptibility of each isolate was determined by the disk diffusion method, as described by the Clinical and Laboratory Standards Institute (CLSI).[10] Minimum inhibitory concentrations (MICs) were calculated using the E-test (AB Biodisk, Solna, Sweden). Isolates with intermediate resistance or resistance were defined as non-susceptible.

2.3. Detection of ESBL- and AmpC-type β-lactamase producers

When suspected based on CLSI screening criteria, ESBL production was confirmed in a phenotypic CLSI confirmatory test.[10] The production of AmpC-type β-lactamase was phenotypically suspected for isolates with reduced susceptibility to ESCs and a negative clavulanic acid synergy test, and for those with a decreased susceptibility to cefotetan or TZP. All of these strains were further tested using boronic acid disks to screen for the presence of AmpC β-lactamase.[4]

2.4. Identification of ESBL types

For isolates obtained between 1999 and 2005, ESBL or AmpC β-lactamase (both are designated as ESBLs from here on, unless otherwise specified) types were identified by analytical isoelectric focusing (with or without clavulanic acid and/or cloxacillin inhibition) and/or sequencing of the respective β-lactamase genes, as described previously.[4,11] Only those TZP-resistant isolates obtained since 2006 were subjected to PCR and sequencing of the specified genes, i.e., TEM, SHV, CTX-M, OXA, DHA, and CMY, carried out in order to characterize the corresponding β-lactamase genes.[12–14]

2.5. Antibiotic policy and antimicrobial consumption data

Beginning in 2002, to reduce the prevalence of ESBL producers, the use of ESCs at our hospital was discouraged, and prescriptions of β-lactam/β-lactamase inhibitor combinations were encouraged instead. The annual amount of each class of antibiotic used in the hospital was determined from the computerized hospital pharmacy database. Antibiotic consumption is expressed as days on the antibiotic per 1000 patient admission days per year (AD).[4,15] Although the antibiotic dosage and administration methods were not thoroughly reviewed for individual cases, treatment guidelines in the Pediatric Dosage Handbook were usually followed.[16] In the case of severe sepsis or febrile illness in neutropenic patients, the maximum recommended dosage of antibiotic was used.

3. Results

3.1. Bacterial isolates

During the 12-year study period, between January 1999 and December 2010, 409 isolates of *E. coli* (n = 170) and *K. pneumoniae* (n = 239) grown from normally sterile body fluids, including blood, ascites, cerebrospinal fluid, and closed pus, were identified based on the records of the microbiology laboratory database.

3.2. Changes in antibiotic consumption

Following the introduction of TZP at our institute in 2001, the use of TZP gradually increased, from 1.3 AD during period 1 to 71.8 AD during period 4 (Table 1). ESC use showed a statistically significant decreasing trend throughout the study period, although a slight increase was observed during the last two periods, from 94.2 AD during period 3 to 102.2 AD during period 4. The patterns of carbapenem usage and total amounts of antibiotics were similar, with an overall nadir during period 3 and then an increasing trend. By contrast, the total consumption of ampicillin/sulbactam and cephamycins remained unchanged.

3.3. ESBL and AmpC β-lactamase detection

Among the 409 isolates, 18.8% (32 of 170) of *E. coli* isolates and 38.5% (92 of 239) of *K. pneumoniae* isolates were determined to be ESBL or AmpC enzyme producers (Table 2). Among the *E. coli* isolates, ESBL prevalence decreased gradually over the first three study periods, from 23.4% (11/47) during period 1 to 17.4% (4/23) during period 2 and 9.3% (5/54) during period 3. However, during period 4, the percentage of ESBL producers among *E. coli* rebounded, reaching 24.2% (8/33). Among *K. pneumoniae* isolates, the prevalence of ESBL producers showed a trend similar to that of *E. coli*; thus, the ESBL prevalence decreased from 56.8% (25/44; period 1) to 26.5% (19/44; period 3), with a rebound to 42.3% (22/

Table 1
Antibiotic usage during the study period

Year	Antibiotic use (days/1000 patient-days/year)					
	Amp/sul	TZP	Cephamycin	ESCs	Carbapenem	Total
1999–2001	69.5	1.3	20.9	144.7	25.3	261.7
2002–2003	74.0	31.6	23.8	145.3	41.3	315.9
2004–2006	64.4	54.4	36.2	94.2	29.3	278.4
2007–2010	61.1	71.8	32.1	102.2	43.8	311.1
p-Value for trend[a]	0.329	<0.001	0.062	0.001	0.093	0.209

Amp/sul, ampicillin/sulbactam; TZP, piperacillin/tazobactam; ESCs, extended-spectrum cephalosporins.
[a] The p-value for trend was evaluated by linear-by-linear association.

Table 2
Frequency of extended-spectrum β-lactamase (ESBL) producers and piperacillin/tazobactam (TZP) resistance among isolates of *Escherichia coli* and *Klebsiella pneumoniae* between 1999 and 2010

Year	*E. coli*			*K. pneumoniae*		
	TZP-resistant isolates, *n* (%)	ESBL[a] producers, *n* (%)	AmpC producers, *n* (%)	TZP-resistant isolates, *n* (%)	ESBL[a] producers, *n* (%)	AmpC producers, *n* (%)
1999–2001	2/47 (4.3)[b]	11/47 (23.4)	0/47 (0)	11/44 (25.0)	25/44 (56.8)	3/44 (6.8)
2002–2003	2/23 (8.7)	4/23 (17.4)	2/23 (8.7)	9/44 (20.5)	19/44 (43.2)	4/44 (9.1)
2004–2006	4/54 (7.4)	5/54 (9.3)	1/54 (1.9)	10/68 (14.7)	18/68 (26.5)	8/68 (11.8)
2007–2010	2/33 (6.1)	8/33 (24.2)	0/33 (0)	14/52 (26.9)	22/52 (42.3)	1/52 (1.9)
Total	13/170 (7.6)	32/170 (18.8)	3/170 (1.8)	50/239 (20.9)	92/239 (38.5)	20/239 (8.4)
p-Value for trend[c]	0.696	0.577	0.839	0.990	0.054	0.493

[a] ESBL and AmpC β-lactamase are jointly designated as ESBLs unless otherwise specified.
[b] The percentage of TZP-resistant strains among the total strains isolated in each time period.
[c] The *p*-value for trend was evaluated by linear-by-linear association.

52) during period 4. The prevalence of AmpC producers showed a decreasing, albeit non-significant, trend (Table 2).

3.4. TZP-resistant *E. coli* and *K. pneumoniae*

Non-susceptibility to TZP was determined in 13 (7.6%) *E. coli* isolates and 50 (20.9%) *K. pneumoniae* isolates. Among the *E. coli* isolates, TZP resistance was stationary over the course of the study: 4.3%, 8.7%, 7.4%, and 6.1% for periods 1 through 4, respectively (*p* for trend = 0.696). Between 2006 and 2008, however, TZP-resistant *E. coli* increased 20.0% (6/30), whereas AmpC producers were not detected (Figure 1). Among *K. pneumoniae*, a drop in TZP resistance from 25.0% to 20.5% and then to 14.7% occurred during periods 1, 2, and 3, respectively, while during period 4, TZP-resistant *K. pneumoniae* increased up to 26.9%.

3.5. Characterization of TZP resistance

Among the 63 TZP-resistant isolates, 13 isolates of *E. coli* and 40 isolates of *K. pneumoniae* were available for further microbiological study, including β-lactamase characterization. The results of antimicrobial susceptibility testing, reported as non-susceptible, were as follows: 82.0% (41/50) for cefotaxime, 74.5% (38/51) for ceftazidime, 26.0% (13/50) for cefepime, 54.7% (29/53) for cefoxitin or cefotetan, and 7.5% (4/53) for imipenem or meropenem.

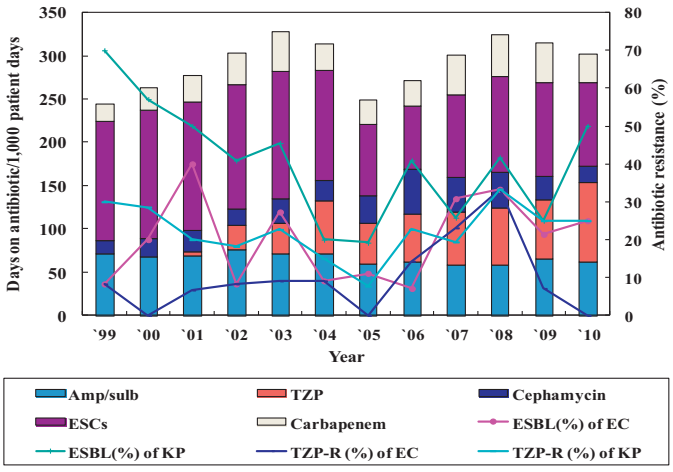

Figure 1. Changes in antibiotic use, extended-spectrum β-lactamase (ESBL) prevalence, and piperacillin/tazobactam resistance over 12 consecutive years (1999–2010). The bar graph represents antibiotic usage, presented as days on antibiotics per 1000 patient-days each year. The solid line shows the prevalence of ESBL and the dotted line shows the prevalence of ESBL among *Escherichia coli* (open triangles) and *Klebsiella pneumoniae* (open squares).

Among the 13 TZP-resistant *E. coli* isolates, only three were positive in a boronic acid test and were confirmed to produce AmpC enzymes, i.e., CMY-1 (*n* = 1), CMY-2 (*n* = 1), and DHA-1 (*n* = 2) (Table 3). Two isolates simultaneously produced both CMY-2 and DHA-1, whereas the remaining 10 isolates did not produce AmpC enzymes. All of the TZP-resistant *E. coli* produced TEM enzymes (TEM-1-like, 10 isolates; TEM-52, two isolates; and TEM-106, two isolates), of which none were IRTs or CMTs. None of the isolates produced ESBLs of the CTX-M family, whereas two SHV-type ESBLs, SHV-2a and SHV-11, were detected.

Among the 40 TZP-resistant *K. pneumoniae* isolates, 15 were positive by boronic acid testing and produced the AmpC enzymes, CMY-1 (*n* = 4) and DHA-1 (*n* = 11) (Table 4). One of the *K. pneumoniae* isolates producing DHA-1 was negative by boronic acid testing. TEM enzymes were produced by 18 isolates: TEM-1-like enzymes were detected in 16 isolates, TEM-52-like in two, and TEM-106-like in one. None of these enzymes were IRT- or CMT-type β-lactamases. CTX-M-type ESBLs were present in only four isolates: CTX-M-12 (*n* = 3) and CTX-M-14 (*n* = 1). Among the TZP-resistant *K. pneumoniae* isolates, SHV-type enzymes were detected in 32: SHV-12-like (*n* = 13), SHV-2a (*n* = 8), SHV-1-like (*n* = 5), SHV-11-like (*n* = 5), and SHV-133 (*n* = 1).

Thus, overall, 10 out of 13 *E. coli* isolates and 24 of 40 *K. pneumoniae* isolates resistant to TZP did not produce either AmpC enzyme or IRTs/CMTs.

4. Discussion

Among *Enterobacteriaceae*, the most prevalent mechanism of acquired resistance to β-lactams is the production of β-lactamases. The penicillinases TEM-1 and SHV-1 hydrolyze only penicillins and narrow-spectrum cephalosporins, whereas these enzymes are inhibited by ESCs and β-lactamase inhibitors, such as clavulanate and tazobactam. However, certain amino acid substitutions confer hydrolytic activity against ESCs under pressure of these antibiotics, and these ESBLs are usually susceptible to β-lactamase inhibitors.[17] Meanwhile, strains producing IRTs, CMTs, or AmpC β-lactamases were found to be generally resistant to inhibitor combinations, and the presence of these enzymes was associated with the increased use of β-lactam/β-lactamase inhibitor combinations.[5,6,9] To decrease the ESBL prevalence at our institute, a change in antibiotic policy was initiated in 2002; thus, TZP rather than ESCs was encouraged for the treatment of Gram-negative bacterial infections, including in febrile neutropenic cancer patients. In a previous study carried out from 1999 through 2005, the overall prevalence at our hospital of ESBL producers among invasive isolates of *E. coli* and *K. pneumoniae* significantly decreased, from 39.8% (41/103) to 22.8% (18/79) (*p* for trend = 0.018).[4] However, beginning in 2005, when the total amount of antibiotic use and the ESBL prevalence had reached a

Table 3
Characterization of piperacillin/tazobactam-resistant *Escherichia coli*

Serial No.	Year of isolation	TZP MIC by E-test (μg/ml)	ESBL or AmpC enzyme characteristics	ESBL confirmatory test	Boronic acid test
1	2004	24	TEM-1	Negative	Negative
2	2006	≥256	TEM-1	Negative	Negative
3	2006	≥256	TEM-1	Negative	Negative
4	2007	≥256	TEM-1, OXA	Negative	Negative
5	1999	32	TEM-1, TEM-52	Positive	Negative
6	2001	64	TEM-1, TEM-52	Positive	Negative
7	2007	>256	TEM-1	Positive	Negative
8	2007	≥256	TEM-106, OXA	Positive	Negative
9	2008	≥256	TEM-106	Positive	Negative
10	2009	≥256	TEM-1, SHV-11	Positive	Negative
11	2002	32	TEM-104, CMY-1	Negative	Positive
12	2004	≥256	TEM-1, CMY-2, DHA-1	Negative	Positive
13	2003	≥256	TEM-1, SHV-2a, DHA-1	Positive	Positive

TZP, piperacillin/tazobactam; MIC, minimum inhibitory concentration; ESBL, extended-spectrum β-lactamase.

minimum, rebounds in the prevalence of ESBL among *E. coli* and *K. pneumoniae* were observed, along with an increase in the amount of total antibiotics, including not only TZP but also ESCs and carbapenems. Therefore, replacement of only the antibiotic class appears to be insufficient to control ESBL prevalence. Instead, broader efforts to decrease overall antibiotic pressure are needed in order to reduce resistant bacteria in highly endemic situations.

In this extended study, a statistically significant change in TZP resistance among *E. coli* and *K. pneumoniae* was not observed, despite a sharp increase in the use of this antibiotic combination. The reasons are currently unknown, but a similar phenomenon was observed by Patterson et al. and described in our previous study.[4,15] However, TZP pressure exerted a greater influence on *K. pneumoniae* than on *E. coli*. Thus, TZP resistance among *K. pneumoniae* reached a nadir at 14.7% during period 3 but nearly doubled, to 26.9%, during period 4, whereas there was no significant increase in TZP resistance among *E. coli* between periods 3 and 4. In our previous study, the revised antibiotic policy was also found to have had a greater impact on *K. pneumoniae* than on *E. coli*. The reason was likewise unclear, but it has been

Table 4
Characterization of piperacillin/tazobactam-resistant *Klebsiella pneumoniae*

Serial No.	Year of isolation	TZP MIC by E-test (μg/ml)	ESBL or AmpC enzyme characteristics	ESBL confirmatory test	Boronic acid test
1	1999	96	TEM-1, SHV-2a	Negative	Negative
2	2000	≥256	TEM-1, SHV-2a	Negative	Negative
3	2004	≥256	SHV-2a	Negative	Negative
4	2006	≥256	TEM-1, SHV-11	Negative	Negative
5	2007	64	SHV-1	Negative	Negative
6	2007	128	SHV-1	Negative	Negative
7	2010	≥256	SHV-11	Negative	Negative
8	2010	≥256	SHV-1	Negative	Negative
9	1999	>128	TEM-1, SHV-12	Positive	Negative
10	2000	≥256	TEM-1, SHV-12	Positive	Negative
11	2001	≥256	TEM-52	Positive	Negative
12	2001	≥256	SHV-2a	Positive	Negative
13	2002	≥256	TEM-1, CTX-M-14	Positive	Negative
14	2003	≥256	SHV-2a	Positive	Negative
15	2003	≥256	TEM-1, SHV-12	Positive	Negative
16	2003	128	TEM-1, SHV-12	Positive	Negative
17	2004	≥256	SHV-12	Positive	Negative
18	2005	16	SHV-12	Positive	Negative
19	2007	≥256	TEM-1, SHV-12, CTX-M-12, OXA, DHA-1	Positive	Negative
20	2007	≥256	CTX-M-12, OXA	Positive	Negative
21	2009	≥256	TEM-1, SHV-2a	Positive	Negative
22	2009	≥256	SHV-2a	Positive	Negative
23	2010	≥256	None	Positive	Negative
24	2000	>128	SHV-2a	Positive	Negative
25	2000	>128	TEM-1, SHV-12	Positive	Negative
26	1999	64	TEM-1, DHA-1	Decreased	Positive
27	2002	48	SHV-12, CMY-1	Decreased	Positive
28	2002	≥256	TEM-1, TEM-52, DHA-1	Decreased	Positive
29	2006	≥256	SHV-11, DHA-1	Decreased	Positive
30	2006	≥256	SHV-11, DHA-1	Decreased	Positive
31	2006	128	SHV-1, DHA-1	Decreased	Positive
32	2003	≥256	SHV-12, CMY-1	Negative	Positive
33	2006	≥256	TEM-1, SHV-1, DHA-1	Negative	Positive
34	2007	≥256	SHV-133, OXA, DHA-1	Negative	Positive
35	2001	≥256	SHV-12, CMY-1	Positive	Positive
36	2002	128	TEM-1, CMY-1	Positive	Positive
37	2004	≥256	TEM-1, DHA-1	Positive	Positive
38	2005	≥256	SHV-12, DHA-1	Positive	Positive
39	2007	≥256	SHV-12, CTX-M-12, OXA, DHA-1	Positive	Positive
40	2008	24	TEM-106, SHV-11, OXA, DHA-1	Positive	Positive

TZP, piperacillin/tazobactam; MIC, minimum inhibitory concentration; ESBL, extended-spectrum β-lactamase.

suggested that these two species differ in their response to changes in antibiotic pressure, with *K. pneumoniae* being more vulnerable to the acquisition and loss of resistance genes.

Resistance to β-lactamase inhibitor combinations including TZP in *E. coli* and *K. pneumoniae* can emerge as a consequence of various mechanisms. Characteristically, AmpC β-lactamases provide resistance to cephamycins as well as ESCs, whereas these enzymes are resistant to inhibition by clavulanic acid.[18] Since *K. pneumoniae* does not possess chromosomal *ampC*, the AmpC enzymes such as DHA-1 and CMY-1 detected in *K. pneumoniae* isolates were plasmid-mediated *ampC* genes. In *E. coli*, although the expression of chromosomal *ampC* is not inducible, in some strains of the bacterium this gene is constitutively overexpressed. The hyperproduction of constitutive chromosomal β-lactamases such as AmpC in *E. coli* and SHV-1 in *K. pneumoniae* can reduce the activity of β-lactam/β-lactamase inhibitor combinations.[19] However, in this study it was not determined whether the AmpC enzymes expressed among *E. coli* were plasmid-mediated or chromosomally mediated. Since plasmid-mediated genes can spread in the hospital setting, the distinction between plasmid and chromosomal enzymes is important in the control of hospital infections.[20] In this study, AmpC enzymes were detected in 23.1% (3/13) of TZP-resistant *E. coli* isolates and 40% (16/40) of TZP-resistant *K. pneumoniae* isolates, with DHA-1 being the most frequently identified enzyme. However, an increasing trend in the prevalence of AmpC producers among *E. coli* and *K. pneumoniae* has yet to be detected.

For the TZP-resistant strains lacking AmpC enzymes, the inoculum effect, β-lactamase hyperproduction, and modifications in the outer membrane protein profile have been suggested to influence the susceptibility of *E. coli* and *K. pneumoniae* to β-lactamase inhibitor combinations.[21] The hyperproduction of TEM-1 β-lactamase due to the presence of either strong promoters or multiple $bla_{\text{TEM-1}}$ copies may result in a loss of susceptibility to β-lactam/β-lactamase inhibitor combinations.[22,23] The hyperproduction of SHV-1 or its ESBL variants has also been described.[24] In our study, most of the TZP-resistant *E. coli* isolates carried the TEM-1 enzyme, although we did not evaluate copy numbers. Future work should focus on the investigation of TEM-1 hyperproducers in *E. coli* and SHV-1 and its variants in *K. pneumoniae* as a cause of TZP resistance. The observed TZP resistance may have been due to the expression of multiple β-lactamases within a single organism, which could lead to variable resistance patterns and might account for the decreased susceptibility to the β-lactamase inhibitors observed in this study.[19]

Among the TZP-resistant strains reported herein, neither IRT nor CMT enzymes were detected; nonetheless, continuous monitoring to guard against the emergence of these types of β-lactamase is needed. Most IRT- and CMT-producing strains are susceptible in vitro to TZP because of the lower activity of their inhibitors under these conditions.[6] Here, the presence of IRT or CMT enzymes was evaluated only as an attempt to explain the occurrence of the TZP-resistant isolates, such that the true impact of the increased use of TZP on the emergence of IRT or CMT could not be evaluated thoroughly. In particular, TZP is not bactericidal against most IRT-producing strains of *E. coli*, especially in the case of high bacterial inoculums. Thus, for the treatment of severe infections caused by IRT-producing organisms, regimens based on relatively high doses might be required to prevent a possible loss of the bactericidal effect.[25]

There are some limitations in this study besides those mentioned above. Specifically, for the characterization of TZP-resistant isolates, only qualitative PCR and sequencing analysis of β-lactamase genes were performed, whereas possible defects in outer membrane proteins such as OmpF and/or OmpC porins were not evaluated. Given that a lack of porins does not significantly affect susceptibility to β-lactams in the absence of a β-lactamase,[19] further studies of outer membrane permeability are needed to clarify the resistance mechanisms. In addition, the methodology of PCR and sequencing used in the detection of candidate β-lactamase genes was limited; further in-depth studies will no doubt shed further light on the mechanisms of TZP resistance.

In conclusion, replacement of only the antibiotic class was insufficient to control resistant bacteria in this highly endemic situation. In this extended study, rebounds in the prevalence of ESBL among invasive isolates of *E. coli* and *K. pneumoniae* were observed, along with an increase in total antibiotic usage, including extended-spectrum β-lactams and TZP. The mechanisms for TZP resistance in this study might include the presence of AmpC producers, multiple β-lactamases in individual organisms of a given isolate, and possible TEM-1 hyperproducers. Despite the increase in TZP usage, the overall prevalence of isolates resistant to TZP did not significantly increase over time especially in the case of *E. coli*, which suggests a relatively higher threshold of resistance acquisition. Continued efforts to decrease overall antibiotic pressure are needed in order to control resistant bacteria in highly endemic situations.

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Conflict of interest: No conflict of interest to declare.

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